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Chapter 5

Imbalance of arginine and ADMA is associated with markers of circulatory failure, organ failure and mortality in shock patients

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Abstract

In shock, organ perfusion is of vital importance because organ oxygenation is at risk. Nitric oxide (NO), the main endothelial-derived vasodilator, is crucial for organ perfusion and coronary patency. The availability of NO might depend on the balance between substrate (arginine) and inhibitor (asymmetric dimethylarginine, ADMA) of NO synthase. Therefore, we investigated the relation of arginine, ADMA and their ratio with circulatory markers, disease severity, organ failure, and mortality in shock patients. In 44 patients with shock (cardiogenic n=17, septic n=27), we prospectively measured plasma arginine and ADMA at ICU admission, APACHE II- (predicted mortality) and SOFA-score, and circulatory markers to investigate their relation.

Arginine concentration was decreased (34·6±17·9 μmol/l) while ADMA concentration was within the normal range (0·46±0·18 μmol/l) resulting in a decrease in the arginine/ADMA ratio. The ratio correlated with several circulatory markers (cardiac index, disseminated intravascular coagulation, bicarbonate, lactate, and pH), APACHE II- and SOFA-score, creatine kinase, and glucose. The arginine/ADMA ratio showed an association (OR:0·976 (95%CI:0·963,0·997), *P*=0·025) and diagnostic accuracy (AUC:0·721 (95%CI:0·560,0·882), *P*=0·016) for hospital mortality, while the arginine or ADMA concentration alone or APACHE II predicted mortality failed to do so.

In conclusion, in shock patients, imbalance of arginine and ADMA is related to circulatory failure, organ failure, disease severity and predicts mortality. We propose a pathophysiological mechanism in shock: imbalance of arginine and ADMA contributes to endothelial and cardiac dysfunction with consequent poor organ perfusion and organ failure, thereby increasing the risk of death.

Introduction

Arginine, the sole nitric oxide (NO) precursor and a semi-essential amino acid, is thought to become essential in shock patients. Sepsis [1] and cardiogenic shock [2] are characterized by low arginine and excessive NO levels. Several studies have tried to boost arginine levels in critically ill patients by supplementing this amino acid alone or in combination with other immuno modulating substances. Results of the studies are controversial. While some showed beneficial or no effects [3], [4], [5], others in fact suggested that arginine might increase the risk of mortality [6], [7]. A possible negative effect of arginine might have been mediated by arginine induced increased NO production by inducible nitric oxide synthase (iNOS) which in turn could have led to detrimental systemic vasodilation [8] or to increased formation of peroxynitrite due to concomitant oxyradical production inducing cellular damage [9]. On the other hand, arginine may have positive effects by enhancing NO mediated microvascular vasodilatation facilitated by endothelial NOS (eNOS) which is crucial for organ perfusion and coronary patency. Likely, NO availability needs to be perfectly balanced [10].

Quantitatively, arginine consumption by NOS is partly determined by the availability of NOS inhibitors, such as asymmetric dimethylarginine (ADMA) which facilitates vasoconstriction and deteriorates endothelial and cardiac function [11]. In critically ill patients, highly elevated levels of ADMA were observed, and high ADMA predicted mortality and correlated with organ severity [12]. Above findings suggest that NO availability might depend on the balance of NOS substrate (arginine) and inhibitor (ADMA). Indeed, in a recent study by our group, low arginine and high ADMA levels reduced cardiac output in rats [13].

Based on previous studies, we hypothesize that an imbalance of arginine and ADMA contributes to poor organ perfusion in patients with shock. Therefore, we investigated the relation between arginine, ADMA and their ratio at admission at the intensive care unit (ICU) and circulatory markers, disease severity, organ failure, and mortality in critically ill shock patients.

Materials and Methods

Patients

This prospective cohort study was conducted in a 20-bed closed format general ICU of a Teaching Hospital in the city of Amsterdam, The Netherlands. The study included adult patients with persistent septic or cardiogenic shock within 24 hours after ICU admission, requiring mechanical ventilation. Other inclusion criteria were: predicted ICU treatment and intention to treat for at least five days. Exclusion criteria were: active massive bleeding, pregnancy, HIV with less than 50 CD4 cells, hematologic malignancy, metastatic malignancy, Child C liver cirrhosis, hepatic coma, and therapeutic hypothermia after cardiac arrest.

For inclusion into the study, shock was defined as persistent hypotension despite adequate fluid resuscitation and the need of dopamine in a dose of more than 6 μg/kg/min and any dose of additional noradrenalin in the presence of perfusion abnormalities, manifest by oliguria, reduced peripheral perfusion and organ dysfunction. Hypotension was defined as a systolic pressure <90 mmHg; oliguria as a urinary output <20 ml/min despite fluid infusion; reduced peripheral perfusion as delta T (difference between core and peripheral temperature) <4°C, skin color not pink, poor capillary refill; and organ dysfunction was defined according to the Sequential Organ Failure Assessment (SOFA) score [(14)]. Septic shock was defined as the form of acute circulatory shock occurring secondary to severe infection [(15)]. Cardiogenic shock is the form of circulatory shock as occurring secondary to heart failure as evidenced by low cardiac output or ejection fraction accompanying cardiac disease (supported by e.g. ECG, echocardiography). To optimize circulation we used fluids in a mixture of crystalloids and colloids (Gelofusine®, B Braun) and dopamine as a first line inotropic and vasopressor agent. Noradrenalin was added if higher doses of dopamine were needed and/or if the patient developed tachycardia. Enoximone was added when cardiac index remained <2·5 L/kg/h in a standard dose of 8 mg/h. Nitroglycerin was used in patients with cardiogenic shock, cardiac ischemia and/or persistent poor peripheral perfusion [(16)]. Fluids were infused in amounts considered to be necessary to restore circulating volume, and to optimize cardiac output and peripheral circulation. Endpoints of circulation treatment were delta T, skin color, capillary refill, blood pressure (target >90mmHg), central venous pressure, cardiac index (target >2·5 l/kg/min, pulse pressure variation (target < 10%) if appropriate and mixed venous oxygen saturation (target >70 mmHg if feasible).

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving patients were approved by the medical ethical committee of the Onze Lieve Vrouwe Gasthuis. Written informed consent was obtained from all patients or their legal representative. Patients were followed until they deceased (non-survivors) or were discharged (survivors) from the hospital.

Physiological and laboratory parameters

Blood samples were taken after inclusion criteria were met in the first 24 hours of ICU admission and were immediately placed on ice and centrifuged. Plasma was pipetted and immediately put in liquid nitrogen and stored at -80°C before analysis. The concentrations of arginine and ADMA were measured by high-performance liquid chromatography on a monolithic column as described previously [(17)], [(18)]. Intra-assay and inter-assay coefficients of variation for both arginine and ADMA were <2% and <3%, respectively. In addition, the arginine/ADMA ratio was calculated. Normal values for arginine and ADMA concentrations are 80±20 μmol/L [(19)] and 0·497±0·063 μmol/L [(17)] respectively.

Laboratory parameters indicating hepatic and renal function (bilirubin, alanine aminotransferase (ALAT), creatinine (clearance) and urea), muscle degradation (creatine

kinase), and acid-base physiology (bicarbonate, pH, and lactate) were analyzed by standard laboratory methods. Diagnosis of disseminated intravascular coagulation (DIC) was done by calculation of the global coagulation test score [20]. At ICU admission, cardiac output was assessed invasively with the Swan Ganz catheter based on the thermodilution method, or non-invasively with NICO (Novametrix Medical Systems, Inc., Wallingford, CT, USA) which continuously measures pulmonary perfusion based on the Fick principle. The NICO was preferred, especially in patients with sepsis, since NICO offers the benefit of continuous monitoring and avoids the risks of invasive monitoring. Cardiac index was calculated by dividing cardiac output with body surface area. Furthermore, severity of illness was scored using the Acute Physiology and Chronic Health Evaluation (APACHE) II system over the first 24-hours of ICU admission [21], and APACHE II predicted mortality was used as reference for the predictive values of arginine, ADMA, and their ratio for mortality [22]. The SOFA score as defined by the Dutch National Intensive Care Evaluation (www.stichting-nice.nl) was measured daily [14].

Statistical analysis

Data were expressed as mean with standard deviation (SD) in case of normally distributed data and as median with interquartile range (IQR) when data were not normally distributed. Normality was tested by Shapiro Wilk Normality Test. Likewise, we used Pearson's correlation and the Spearman rank correlation coefficient to determine whether clinical and biochemical variables were significantly related to arginine, ADMA, and their ratio. Predictors of mortality were studied by calculation of the odds ratio (OR) in a logistic regression model with one variable (arginine, ADMA, arginine/ADMA ratio or APACHE II predicted mortality). Our sample size did not permit a multiple regression analysis. Receiver operating characteristic (ROC)-curves were estimated using the non-parametric method to further evaluate the association between arginine, ADMA, arginine/ADMA ratio, and APACHE II predicted mortality and hospital mortality. The area under the curve (AUC) was calculated to determine the accuracy of arginine, ADMA, arginine/ADMA ratio, and APACHE II predicted mortality as predictor of hospital mortality. The further the curve lies above the reference line and the higher the AUC, the more accurate the test. Coordinates of the curve were examined across the full range of potential arginine/ADMA cut-off values in an attempt to select an optimal arginine/ADMA cut-off that properly balanced the needs of sensitivity and specificity. Furthermore, positive and negative predictive values of the arginine/ADMA cut-off were calculated. Chi-square test was used to analyze the difference in mortality between patients with an arginine/ADMA ratio below the cut-off and an arginine/ADMA ratio's above the cut-off value. A *P*-value of <0·05 (2-tailed) was considered as statistically significant. Statistical analysis was performed with SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Patients

Patient characteristics and biochemical values are presented in Table 1. Of the 44 shock patients, 27 had septic and 17 had cardiogenic shock. At admission, mean APACHE II-score was 26·2±7·4 and SOFA-score was 10·0±3·0. Mean levels of arginine and ADMA were 34·6±17·9 µmol/l and 0·46±0·18 µmol/l respectively, and mean arginine/ADMA was 83·2±42·5. The levels of arginine and ADMA, the arginine/ADMA ratio, APACHE II- and SOFA-score and cardiac index did not differ significantly between septic and cardiogenic shock patients (arginine: 32·2±15·9 and 38·4±20·7, *P*=0·27; ADMA: 0·43±0·16 and 0·50±0·20, *P*=0·19; arginine/ADMA: 79·9±33·7 and 88·4±54·4, *P*=0·57; APACHE II-score: 26·5±7·7 and 25·6±7·2, *P*=0·69; SOFA-score: 9·9±3·2 and 10·3±1·7, *P*=0·89; cardiac index: 2·5±1·1 and 2·0±1·0, *P*=0·15, respectively).

Table 1 Patient characteristics and biochemical values

	n (%)	mean or median[a]	SD or IQR[a]
Demographics			
Gender: female/male (%)	21/23 (47·7/52·3)		
Age (year)		65·7	13·8
Height (cm)		172·5	8·9
Weight (kg)		75·3	17·4
Clinical assessment			
ICU admission type: medical/surgical (%)	30/14 (68·2/31·8)		
ICU stay (days)[a]		5·9	3·7 to 9·3
ICU mortality (%)	8 (18·2)		
Hospital mortality (%)	16 (36·4)		
APACHE II-score		26·2	7·4
APACHE II predicted mortality		0·56	0·25
SOFA-score		10·0	3·0
Cardiac index (L/min/m^2)		2·3	1·1
Shock: septic/cardiogenic (%)	27/17 (61·4/38·6)		
Site of infection (%)			
Lung	13 (29·5)		
Abdomen	8 (18·2)		
Urogenital	1 (2·3)		
Other	5 (11·4)		
None (cardiogenic shock)	17 (38·6)		

Dose of vasoactive agents at 1st day		
Dopamine (µg/kg/min)	8·1	2·8
Noradrenalin (µg/kg/min)	0·04	0·04
Nitroglycerin (µg/kg/min)	0·39	0·18
Laboratory measurements		
Bilirubine (µmol/L)	14·8	11·3
ALAT (U/L)[a]	29·0	15 to 67
Creatinine (µmol/L)[a]	124·5	87·3 to 167·8
Creatinine clearance[a]	52·3	32·2 to 67·2
Urea (mmol/L)[a]	9·4	7·1 to 17·4
Creatine Kinase (U/L)[a]	99·5	60·3 to 458·5
DIC	2·8	1·2
Bicarbonate (mmol/L)	18·7	5·9
Lactate (mmol/L)[a]	3·6	1·9 to 6·4
pH	7·3	0·13
Glucose (mmol/L)	9·5	5·0
Arginine (µmol/l)	34·6	17·9
ADMA (µmol/l)	0·46	0·18
Arginine/ADMA	83·2	42·5

ICU, intensive care unit; APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment; ALAT, alanine aminotransferase; DIC, disseminated intravascular coagulation; ADMA, asymmetric dimethylarginine.

Arginine, ADMA and their ratio in shock patients

Arginine correlated negatively with ICU length of stay, APACHE II predicted mortality, SOFA-score, and urea. ADMA (tended to) correlate(d) positively with ALAT, urea, and glucose, and negatively with pH. The arginine/ADMA ratio correlated with markers of circulation (cardiac index (Fig. 1A), bicarbonate (Fig 1B), lactate score (Fig 1C), and pH (Fig. 1D)), and negatively with APACHE II and SOFA-score (Table 2). The arginine/ADMA ratio also correlated positively with creatine kinase and glucose (Table 2).

Predictors of hospital mortality

In a logistic regression model, the arginine/ADMA ratio predicted hospital mortality (OR: 0·980 (95 % confidence interval (CI): 0·963, 0·997), *P* = 0·025), while arginine, ADMA and APACHE II predicted mortality were not significantly related to hospital mortality (OR: 0·976 (95 % CI:0·940, 1·013), *P* = 0·205; OR: 40·9 (95 % CI:0·867, 1930), *P* = 0·059; OR: 3·47 (95 % CI: 0·267, 45·2), *P* = 0·34, respectively), although the relation with ADMA tended to significance (Table 3). The ROC curves in Figure 2 reveal that the arginine/ADMA ratio (AUC: 0·721 (95 % CI: 0·560, 0·882) *P* = 0·016, Fig 2C) provides better diagnostic accuracy

to predict mortality compared to arginine (AUC: 0·621 (95 % CI: 0·443, 0·798), *P* = 0·188) (Fig 2A), ADMA (AUC: 0·706 (95 % CI: 0·533, 0·880), *P* = 0·024) (Fig 2B), and APACHE II predicted mortality (AUC: 0·583 (95 % CI: 0·401, 0·765), *P* = 0·367) (Fig 2D). The optimal arginine/ADMA cut-off value was 93.4 (sensitivity: 0·875, specificity: 0·571) with a positive predictive of 0·54 (95 % CI: 0·36, 0·71) and a negative predictive value of 0·89 (95 % CI: 0·67, 0·97) (Table 4). Hospital mortality was significantly higher in patients with an arginine/ADMA ratio below the cut-off value compared to patients above the cut-off value (14/24 *vs*. 2/18, Chi-square *P* = 0·004).

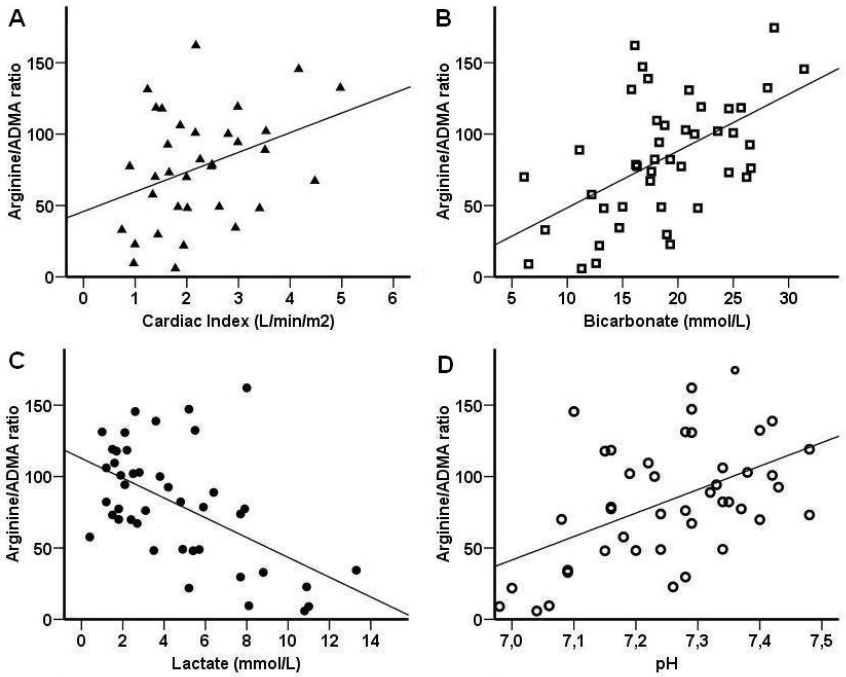

Figure 1 The arginine/ADMA ratio correlates with markers of circulation.
Cardiac Index: r^2 = 0·132; bicarbonate: r^2 = 0·305; lactate: r^2 = 0·218; pH: r^2 = 0·248.
ADMA – asymmetric dimethylarginine.

Table 2 Correlations of arginine, ADMA and arginine/ADMA ratio and clinical and biochemical variables

	Arginine		ADMA		Arginine/ADMA	
	r	*P*	r	*P*	r	*P*
ICU stay[a]	-0·412	0·027	-0·040	0·794	-0·070	0·652
APACHE II-score	-0·198	0·302	0·086	0·581	-0·314	0·038
APACHE II predicted mortality	-0·386	0·010	-0·062	0·688	-0·256	0·093
SOFA-score	-0·391	0·009	0·160	0·301	-0·362	0·016
Cardiac index	0·176	0·320	-0·279	0·110	0·364	0·034
Bilirubine	-0·004	0·978	0·261	0·087	-0·154	0·317
ALAT[a]	-0·126	0·516	0·303	0·045	-0·790	0·612
Creatinine[a]	-0·011	0.944	-0·027	0·863	-0·021	0·891
Creatinine clearance[a]	0·044	0.775	0·005	0·974	0·123	0·427
Urea[a]	-0·436	0.018	0·285	0·061	-0·095	0·540
Creatine Kinase[a]	-0·319	0.092	-0·048	0·755	0·371	0·013
Bicarbonate	0·156	0.420	-0·176	0·253	0·552	<0·001
pH	0·125	0.518	-0·380	0·011	0·498	0·001
Lactate[a]	0·070	0.717	0·267	0·084	-0·467	0·002
DIC	-0·097	0.625	0·245	0·118	-0·409	0·007
Glucose	-0·220	0.151	0·395	0·008	0·337	0·025

ADMA, asymmetric dimethylarginine; ICU, intensive care unit; APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment; ALAT, alanine aminotransferase; DIC, disseminated intravascular coagulation. Data are expressed as Pearson's correlation or as [a]: Spearman rank correlation coefficient.

Table 3 Risk factors for hospital mortality

	OR	95 % CI	*P*
Arginine	0·976	0·940, 1·013	0·205
ADMA	40·9	0·867, 1930	0·059
Arginine/ADMA	0·980	0·963, 0·997	0·025
APACHE II predicted mortality	3·47	0·267, 45·2	0·34

ADMA, asymmetric dimethylarginine; APACHE, acute physiology and chronic health evaluation.

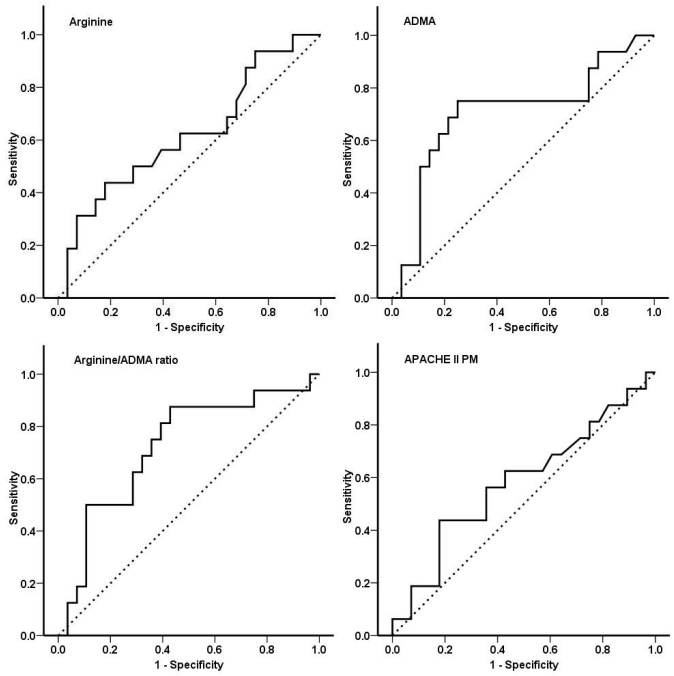

Figure 2 ROC curves of arginine, ADMA, arginine/ADMA and APACHE II predicted mortality. ADMA – asymmetric dimethylarginine; APACHE II PM – acute physiology and chronic health evaluation II predicted mortality; ROC – receiver operating characteristic.

Table 4 – Predictive values of the arginine/ADMA ratio for hospital mortality

	Arginine/ADMA ratio		
	< 93·4	≥ 93·4	*p**
Survivors	12	16	0·004
Non-survivors	14	2	
Positive predictive value	0·54 (95 % CI: 0·36, 0·71)		
Negative predictive value		0·89 (95 % CI: 0·67, 0·97)	

*Chi-square test
ADMA, asymmetric dimethylarginine.

Discussion

The present observational cohort study in critically ill patients with shock shows that an imbalance of arginine and ADMA at ICU admission is associated with circulatory failure, severity of disease and organ failure, and also predicts mortality. The imbalance of arginine and ADMA in this study was mainly the result of arginine depletion because ADMA levels were within the normal range. Furthermore, the arginine/ADMA ratio was related to all markers of circulation while arginine and ADMA separately were not. In addition, the ratio showed a stronger association and better diagnostic accuracy for hospital mortality in our study while the arginine or ADMA concentration alone failed to do so. It therefore seems that the balance of arginine and ADMA might be important for proper micro- and macro-circulation and not their individual concentrations. When arginine is depleted, a normal ADMA concentration may be relatively high. In the present shock patients, this imbalance between the NO substrate (arginine) and inhibitor (ADMA) may result in poor organ perfusion.

Since poor organ perfusion is a main cause of organ failure, and both arginine and ADMA influence endothelial and cardiac function [11], the present observations support the role of an imbalance between the NO substrate and inhibitor in the pathophysiological mechanism of circulatory failure, organ failure and mortality in patients with shock. We propose a mechanism in which a combination of reduced flow to the organs and diminished microcirculation within these organs as a result of an imbalance between arginine and ADMA will end up in organ failure and an increased risk of death in shock patients. The associations with mortality and disease severity in septic patients with low arginine/ADMA ratios as reported in the literature [23], [24] and the associations found in our study support this mechanism. The magnitude of the OR of 0·98 can be illustrated by an example: for a patient with an arginine/ADMA ratio of 80, the odds of dying are 1·50 ($1/(0{\cdot}98^{20})$) times higher than for a patient with an arginine/ADMA ratio of 100.

The present results are in accordance with an experimental study done by our group in which a low arginine/ADMA ratio reduced cardiac output and diminished flow in the microcirculation of major organs in animals [13]. In addition, exogenous arginine increased coronary blood flow and restored perfusion in the ischemic areas in the endotoxin-treated hearts [25], and increased cardiac output in sepsis [26], [27]. On the other hand, safety of arginine administration remains controversial because two studies showed increased mortality rates with supplementation of this amino acid in sepsis [6], [7]. However, both studies used immunonutrition with low arginine formula which makes it difficult to attribute this effect to a single nutrient. Furthermore, the strength of the design of one of these studies [6] can be doubted as different routes of feeding were used and the statistical significance was quite thin since one more deceased subject would have made the difference non-significant. The influence of ADMA is supported by studies in which administration of NOS inhibitors reduced cardiac output in healthy volunteers [28] and in septic shock patients [29], and reduced coronary flow [25] and induced local ischemia [25] in

endotoxemic rats. Furthermore, a study with cardiogenic shock patients suggested that ADMA can regulate flow to the lung as the NOS inhibitor was associated with pulmonary capillary wedge pressure and with both systolic and diastolic pulmonary artery pressures [2]. Nevertheless, NOS inhibitors have been proposed as a treatment for the overproduction of NO in sepsis [29]. The hypothesis underlying the use of NOS inhibitors is that the increased production of NO by iNOS in sepsis contributes to hypotension and multiple organ dysfunction [30]. However, results of these experiments are conflicting [26], [29], [31]. Moreover, a non-selective NOS inhibitor increased mortality rate of septic shock patients which was associated with decreased cardiac output and heart failure [31]. Overcorrection of vascular tone by the non-selective NOS inhibitor might have hampered organ perfusion resulting in myocardial and vascular dysfunction.

The observed low arginine/ADMA ratio in patients with high severity of disease and organ failure, and low cardiac index may be the result of decreased production or increased degradation of arginine and/or increased production or decreased clearance of ADMA, or both of these. In our patients, ADMA levels (0·44±0·15 µmol/L) on ICU admission were not elevated compared to normal values (0·5 µmol/L [17]), while arginine levels were decreased (32·2±16·6 compared to 80 µmol/L in healthy subjects [19]). Similar results were found in a study by Mittermayer *et al*. in healthy males after injection of Eschericia coli endotoxin; arginine concentration decreased while ADMA levels were not affected, resulting in a decline of the arginine/ADMA ratio [32]. These observations suggest that the effect of the arginine/ADMA ratio on clinical outcome can mainly be the consequence of low arginine levels. Arginine depletion in shock is probably the result of diminished production of arginine [33] or its precursor citrulline [1].

The limitations of our study need to be addressed. Unfortunately, we could not measure NO or its oxidation products nitrite and nitrate. NO is highly reactive and has a short half-life (< 0.1 sec in human circulation) [34]. Furthermore, plasma levels of NO oxidation products are influenced by several factors such as endogenous NO synthesis, dietary intake and excretion, and clinical and therapeutic interventions. Therefore, the production of NO, nitrite and nitrate may not be reliably assessed in shock patients. Secondly, cardiac output was not uniformly measured, because we used both the pulmonary artery catheter and the NICO for cardiac output measurement. This choice is based on the absence of clinical evidence that the use of a pulmonary artery catheter reduces morbidity or mortality, that a continuous measurement as provided by NICO improves optimization of the circulation at the bedside, and reduces the risk of infection in septic patients [35]. However, cardiac output is only one of the markers of circulation. We also found a significant relation with other markers of circulation such as DIC score, bicarbonate, lactate and metabolic acidosis. Finally, because of the small sample size and the observational design of our study, it is not possible to draw conclusions about the cause-effect relationship of our results.

In conclusion, the main finding of the present observational study is that an imbalance of arginine and ADMA at ICU admission is associated with circulatory failure, organ failure and mortality in patients with septic or cardiogenic shock. In the present study, the imbalance was caused by arginine depletion, while ADMA concentrations were normal. However, the ratio was related to markers of circulation and outcome, while arginine and ADMA levels alone were not. These results support our hypothesis that an imbalance of arginine and ADMA, being substrate and inhibitor of NO synthase respectively, might contribute to endothelial and cardiac dysfunction with subsequent poor organ perfusion and organ failure thereby increasing the risk of mortality. Future studies should focus on the role of arginine supplementation or ADMA removal in a specific subgroup of early shock with persisting circulatory failure.

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